Drug Design, Development and Therapy

Dovepress
Taylor & Francis Group

Open Access Full Text Article

ORIGINAL RESEARCH

Integrating Therapeutic Drug Monitoring and Metabolomics to Explore Interindividual Variability in Lamotrigine Response in Epilepsy

Xiuwei Shen[1,*], Ruyi Jin[2,*], Ningxia Xu[2], Yuqing Miao[2], Zhenfei Zheng[2], Jianxing Wang[2], Minjie You[2], Feiyan Lin[3], Lufeng Hu[2]

[1]Department of Pharmacy, The Third Affiliated Hospital of Wenzhou Medical University, Wenzhou, People's Republic of China; [2]Department of Pharmacy, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou, People's Republic of China; [3]Clinical Research Center, The Second Affiliated Hospital of Wenzhou Medical University, Wenzhou, People's Republic of China

*These authors contributed equally to this work

Correspondence: Feiyan Lin, Clinical Research Center, The Second Affiliated Hospital of Wenzhou Medical University, Lonwan District, Wenzhou, 325024, People's Republic of China, Tel +86-577-55579706, Email singlelin@yeah.net; Lufeng Hu, Department of Pharmacy, The First Affiliated Hospital of Wenzhou Medical University, Nanbaixiang Street, Ouhai District, Wenzhou, 325000, People's Republic of China, Tel +86-577-55579706, Email hulufeng79@sina.com

Objective: Lamotrigine (LTG) is a first-line antiseizure medication, yet substantial interindividual variability in therapeutic response remains a major clinical challenge. This study integrated therapeutic drug monitoring (TDM) and metabolomics to explore pharmacokinetic and metabolic factors associated with LTG efficacy in patients with epilepsy.

Methods: A total of 141 epilepsy patients receiving LTG therapy were retrospectively enrolled and classified as responders or non-responders by 6-month seizure frequency reduction. LTG plasma concentrations were measured using HPLC–DAD. Untargeted UPLC–MS/MS metabolomics and multivariate analysis were performed on serum from 10 responders and 10 non-responders with LTG >5.15 mg/L, with differential amino acids quantified. Differential metabolites were further quantified, with a focus on amino acids.

Results: Mean LTG plasma concentration was significantly higher in responders (7.55 ± 4.57 mg/L) than non-responders (4.53 ± 2.94 mg/L; $P = 0.001$). Receiver operating characteristic (ROC) curve analysis suggested 5.15 mg/L as the optimal cut-off for discriminating responders from non-responders, within the conventional ILAE reference range (2.5–15 mg/L). Concomitant use of valproic acid (VPA) increased LTG exposure, was more prevalent among responders, and enabled seizure control at lower LTG doses. Metabolomic analysis in the high-concentration subgroup revealed a distinct amino acid profile in responders, characterized by higher proline and lower lysine and cystine ($P < 0.05$). These changes suggest reduced oxidative stress and improved neuronal stability in responders.

Conclusion: Higher LTG plasma concentrations were associated with better seizure control, and VPA co-administration enhances LTG exposure and efficacy. The distinct amino acid profile in high-concentration responders suggests amino acid metabolism and redox balance may modulate LTG efficacy.

Keywords: lamotrigine, epilepsy, therapeutic drug monitoring, metabolomics, amino acid

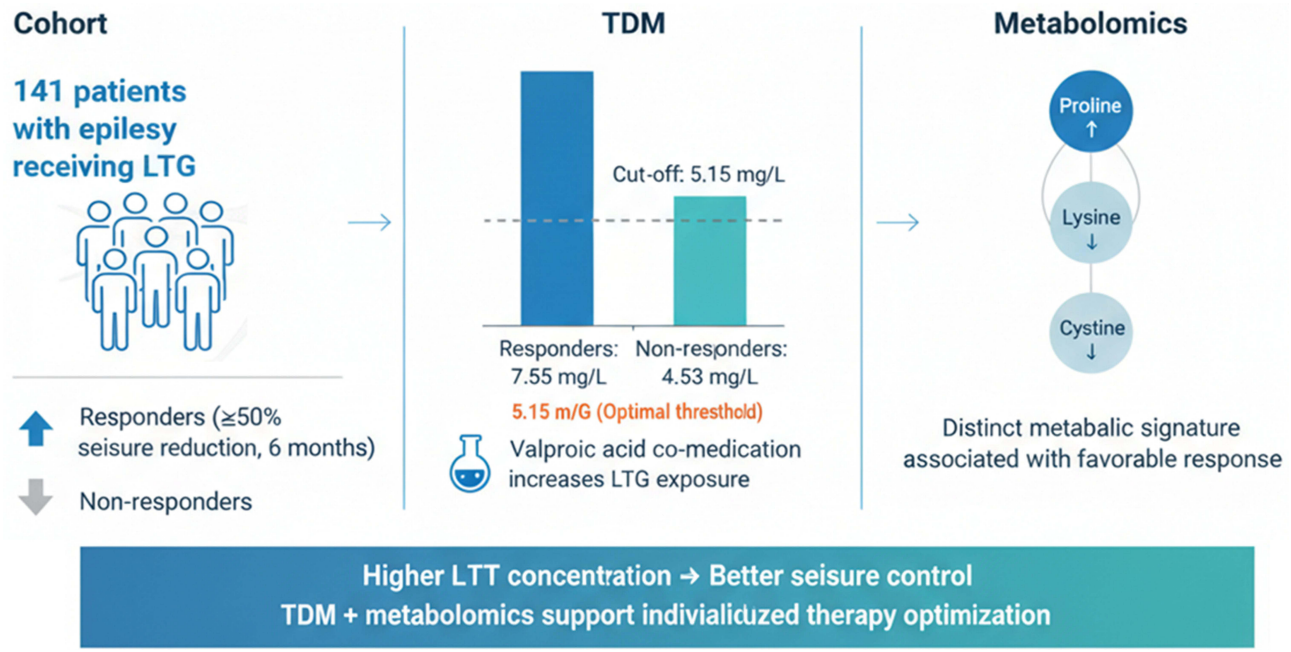
Introduction

Epilepsy is a synchronous neuronal discharge in the brain. Between 1990 and 2021, the age-standardized global prevalence of epilepsy increased by 10.8%, primarily driven by a rising incidence of secondary epilepsy cases.[1,2] Marked geographical disparities persist, with over 80% of individuals with epilepsy living in low- and middle-income countries.[3] Consequently, epilepsy remains a major global public health challenge, affecting more than 50 million people worldwide as of 2021.[4,5]

According to the WHO, up to 70% of individuals with epilepsy can achieve seizure freedom with appropriate use of antiseizure medications.[6] LTG is widely used for the treatment of both focal and generalized seizures through multiple mechanisms, including inhibition of voltage-gated sodium channels and modulation of neurotransmitter release.[7,8] Since its

https://doi.org/10.2147/DDDT.S593268
Received: 4 January 2026
Accepted: 23 April 2026
Published: 5 May 2026

Graphical Abstract



introduction in 1989, LTG's safety and efficacy have been well established in clinical practice. Large randomized controlled trials, such as the SANAD study, demonstrated that LTG is superior to valproate with regard to time to treatment failure in idiopathic generalized epilepsy, particularly when tolerability and long-term outcomes are considered. These findings have solidified LTG's status as the preferred ASM for women of childbearing potential.[9] Updated guidelines from the International League Against Epilepsy (ILAE) comprehensively reviewed the evidence on ASM efficacy and classified LTG as a Level A recommendation for initial monotherapy in more than one seizure type.[10]

The pharmacokinetics of LTG are influenced by multiple factors, including genetic polymorphisms (eg., in UGT1A4 and ABC transporter genes),[11,12] concomitant medications (eg., enzyme inhibition by valproate or induction by carbamazepine), as well as patient-specific characteristics such as age, and hepatic or renal function. These variables contribute to substantial interindividual variability in plasma LTG concentrations.[13] The 2008 ILAE guideline proposed a reference therapeutic range for LTG of 2.5–15 mg/L.[14] However, clinical observations reveal considerable overlap between effective and toxic concentrations. Some patients achieve complete seizure control at concentrations below 2.5 mg/L, while others continue to experience frequent seizures despite concentrations exceeding 15 mg/L. Notably, signs of toxicity have been reported in 24% of patients at plasma concentrations of 10–15 μg/mL, rising to 59% when concentrations exceed 20 μg/mL.[15]

Therefore, defining an optimal, individualized therapeutic range remains a key clinical challenge in epilepsy management. Further elucidation of target LTG concentrations across distinct patient populations, as well as understanding the metabolic mechanisms underlying interindividual variability in drug response, are essential for advancing personalized epilepsy therapy. This study aims to investigate the relationship between LTG plasma concentrations and clinical efficacy, and to employ metabolomic profiling to identify potential biomarkers associated with differential therapeutic responses to LTG, thereby providing a scientific basis for precision dosing in clinical practice.

Method

Ethics Statement and Data Collection

This retrospective observational study consecutively enrolled patients with epilepsy who received LTG therapy and underwent therapeutic drug monitoring at our institution between January 2021 and November 2023. The study protocol

was approved by the Ethics Committee of the First Affiliated Hospital of Wenzhou Medical University (Approval No. KY2023-R260). As only anonymized clinical data were used and no interventions were performed, the requirement for individual informed consent was waived by the ethics committee. All patient identifiers were removed prior to data analysis to ensure confidentiality, in accordance with the principles of the Declaration of Helsinki and the relevant Chinese regulations governing biomedical research involving human participants.

Inclusion and Exclusion Criteria

Patients were included only if they had maintained a stable LTG dosage for at least 3 months to reach steady state. The treatment response was then monitored over a subsequent 6-month period.

Inclusion Criteria

(1) Diagnosis of epilepsy confirmed according to the 2017 International League Against Epilepsy (ILAE) criteria; (2) Age ≥ 12 years; (3) Receiving LTG as monotherapy or adjunctive therapy; (4) Availability of complete LTG plasma concentration monitoring records at our institution; (5) Normal hepatic and renal function; (6) Regular LTG intake for more than six months with good adherence to follow-up visits.

Exclusion Criteria

(1) LTG prescribed for non-epileptic conditions (eg., bipolar disorder); (2) Undetectable LTG plasma concentrations; (3) Coexisting central nervous system tumors or neurodegenerative diseases; (4) Severe cardiac, hepatic, or renal insufficiency; (5) Incomplete clinical medication records.

Efficacy Assessment and Data Collection

Treatment efficacy was evaluated according to established criteria. After LTG reached the target maintenance dose, patients were followed for at least six months, and response was assessed based on changes in seizure frequency. Complete seizure freedom or a reduction in seizure frequency of ≥50% was classified as effective (responders), whereas <50% reduction or an increase was defined as ineffective (non-responders).[16,17]

Demographic information-including age, sex, height, weight, and medical history-was extracted from the Hospital Information System (HIS). Medication details included daily LTG dose, dosing frequency, and comprehensive information on all concomitant drugs. Laboratory tests comprised a complete blood count, liver and renal function tests, and other relevant biochemical parameters, such as lipid profile and blood glucose levels.

LTG Concentration Measurement and Analysis

Plasma LTG concentrations were quantified using high-performance liquid chromatography with diode-array detection (HPLC–DAD). The chromatographic conditions were as follows: an Agilent HC-C18 column (4.6 mm × 150 mm, 5 μm) maintained at 35°C; isocratic elution with a mobile phase of 5 mM potassium dihydrogen phosphate–acetonitrile (15:85, v/v); flow rate of 1.0 mL/min; and detection wavelength set at 246 nm. Detailed method validation procedures have been published previously.[18]

LTG plasma concentrations were correlated with clinical outcomes, and the optimal therapeutic cut-off concentration for seizure control was determined using receiver operating characteristic (ROC) curve analysis.

Serum Metabolite Analysis

Based on the efficacy assessment, serum samples from patients classified as either LTG-effective or LTG-ineffective were selected. Samples were centrifuged at 3000 rpm for 10 minutes at 4°C, and the resulting supernatants were collected and stored at −80°C until non-targeted metabolomic analysis by ultra-performance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS). Metabolomic profiling was performed using M1 Scan combined with Selected Ion Recording (SIR) mode.

Sample Preparation and Quality Control

Fifty microliters (50 μL) of serum were mixed with 200 μL of acetonitrile for protein precipitation, vortexed vigorously for 30 seconds, and then centrifuged at 12,000 rpm for 5 minutes at 4°C. The supernatant was collected, and a 2 μL aliquot was injected into the analytical system.

Quality control (QC) samples were prepared by pooling equal volumes from 10 randomly selected serum samples. QC samples were analyzed at regular intervals throughout the analytical sequence to monitor instrument stability and ensure data quality.

Chromatographic and Mass Conditions

Chromatographic separation was performed on a Waters ACQUITY UPLC BEH C18 column (2.1 mm × 100 mm, 1.7 μm particle size, 130 Å pore size). The column temperature was maintained at 30°C, and the autosampler was set to 4°C. The mobile phases consisted of water/formic acid (99.9:0.1, v/v; solvent A) and acetonitrile/formic acid (99.9:0.1, v/v; solvent B). The flow rate was 0.4 mL/min. A gradient elution program (detailed in Supplement Table 1) was applied, with a total run time of 20 minutes per sample and an injection volume of 2 μL.

Mass spectrometric detection was performed using a Waters Q-TOF Premier mass spectrometer equipped with an electrospray ionization (ESI) source operating in positive-ion mode. The source temperature was set to 120°C. Key MS parameters were as follows: mass scan range, m/z 50–1000; data acquisition rate, 0.2 s per scan; nitrogen desolvation gas flow, 150 L/h; capillary voltage, 1.0 kV; desolvation temperature, 600°C; and desolvation gas flow, 1000 L/h.

Screening of Differential Metabolites

Data were acquired using the UPLC–MS system. Raw data files were converted into a compatible format and processed with MS-DIAL software (version 4.60) for peak detection, alignment, and metabolite identification. The processed M1 Scan and SIR data were imported into SIMCA-P software (version 13.0). Partial Least Squares Discriminant Analysis (PLS-DA) was applied to construct a classification model distinguishing the LTG-effective group from the LTG-ineffective group. Variable Importance in Projection (VIP) scores were calculated for all metabolites. Metabolites with VIP > 1 and a statistically significant difference ($P < 0.05$) were regarded as potential differential biomarkers. The top 20 metabolites ranked by VIP value were selected for further identification.

Metabolite identification was carried out by matching the observed m/z values, retention times, and adduct forms (eg., $[M+H]^+$) with reference data. Tentative metabolite identities were subsequently verified by comparing MS^1 (precursor ion) and MS^2 (fragment ion) spectra against integrated databases, including METLIN, MassBank, MoNA, and HMDB (version 8.0).

Metabolites Quantification

To further validate the differential metabolites, quantification was performed using a Waters UPLC-MS/MS system equipped with a BEH C18 chromatographic column. The chromatographic conditions involved gradient elution with 0.1% ammonium hydroxide and acetonitrile as the mobile phases. Mass spectrometry was operated in electrospray ionization positive mode with multiple reaction monitoring (MRM). Data acquisition and processing were performed using MassLynx software (version 4.1). After data acquisition, peak areas of the quantified amino acids were normalized. Group differences between the LTG-effective and LTG-ineffective groups were assessed using independent-samples *T* test in SPSS software (version 26.0).

Predictive Model for Treatment Response

Additionally, four commonly used machine learning methods—support vector machine (SVM), back propagation artificial neural network (BP-ANN), XGBoost, and random forest (RF)—were used to predict antiepileptic treatment efficacy. The results showed that RF had the best classification performance, so it was selected for modeling (Supplement Figure 1).

To evaluate the generalization performance of the RF model, the dataset was split into training and test sets. Hyperparameters such as "n_estimators" and "max_depth" were optimized by grid search. Information gain and feature importance were used to quantify the contribution of each variable.

To examine whether adding amino acids improves prediction of treatment responsiveness, we built models on two datasets: one including LTG concentration, complete blood count, and serum biochemistry, and another additionally incorporating amino acids. This comparison aims to enhance the clinical utility of the prognostic model, both with standard clinical variables alone and with amino acids information. Model evaluation followed procedures described in the published literature.[19]

Results

Patient Characteristics

A total of 152 patients with epilepsy were initially enrolled in this study. Eleven patients were excluded due to follow-up duration of less than six months. Ultimately, 141 patients were included in the efficacy analysis: 55 were classified as responders (effective), defined as having ≥50% reduction in seizure frequency or being seizure-free over the past six months, and 86 as non-responders (ineffective), defined as <50% reduction in seizure frequency, including no change or an increase over the same period. Among the non-responder, 50 patients had their treatment regimens modified due to inadequate therapeutic response.

There were no significant differences between the two groups in terms of age, sex, height, or body weight (all $P > 0.05$). However, the daily LTG dose differed significantly between groups ($P < 0.05$), suggesting an association between LTG dosage and treatment efficacy (Table 1).

Analysis of Antiseizure Medications

Both groups included patients receiving concomitant antiseizure medications, such as sodium valproate (VPA) and levetiracetam. Overall, the rate of polytherapy did not differ significantly between groups; however, the proportion of patients on combination therapy was higher in the responder group (70.9%). Notably, the use of VPA in combination with LTG was significantly more common in responders (41.8%) than in non-responders (15.1%).

In the responder group, patients receiving LTG + VPA had a lower mean daily LTG dose compared to those on LTG monotherapy, which exhibited significantly higher LTG plasma concentrations (10.67 ± 4.45 mg/L vs. 4.88 ± 2.66 mg/L), indicating a strong inhibitory effect of VPA on LTG metabolism.

In contrast, LTG monotherapy was more prevalent in the non-responder group (39.5%), and both the average daily LTG dose and plasma concentration in these patients were lower than those observed in monotherapy-treated responders. Even when used at a lower daily dose, LTG co-administered with VPA resulted in higher plasma concentrations than LTG monotherapy.

As reported in the literature, VPA-acting as a hepatic enzyme inhibitor—suppresses the glucuronidation pathway of LTG metabolism in the liver, leading to an approximately 2.23-fold increase in LTG plasma concentrations.[20] These findings further support that concomitant VPA use elevates LTG plasma levels and enhances its antiseizure efficacy. Detailed data on dosing regimens and corresponding LTG plasma concentrations across different treatment groups are presented in Table 2.

Table 1 Comparison of Basic Conditions Between the Effective and Ineffective Treatment Groups of LTG in 141 Epilepsy Patients

Parameters	Effective (n=55)	Ineffective (n=86)
Age (years)	34.87±14.18	31.88±12.90
Gender (Male)	27(49.1%)	32(37.2%)
Gender (Female)	28(50.9%)	54(62.8%)
Height (cm)	166.53±7.80	164.87±9.16
Weight (kg)	62.59±11.70	58.61±11.57
Daily dosage (mg)	245.45±117.17	187.50±88.84*

Note: *<0.001.

https://doi.org/10.2147/DDDT.S593268

Table 2 Combination Therapy Analysis of 141 LTG-Treated Epilepsy Patients

Drug	Effective Group (n=55)			Ineffective Group (n=86)		
	Number	Daily Dose (mg)	Concentration (mg/L)	Number	Daily Dose (mg)	Concentration (mg/L)
LTG	16(29.1%)	256.25±134.01	4.88±2.66	34(39.5%)	169.85±85.00	3.35±2.53
LTG+VPA/	20(36.4%)	230.00±95.15	10.67±4.45*	9(10.5%)	125.00±66.14	5.21±2.35
LTG+LEV	13(23.6%)	238.46±138.68	5.49±2.70	21(24.4%)	222.62±88.00	4.68±2.96
Other	9 (11.0%)	266.67 ± 89.25	7.10 ± 2.40	23 (25.6%)	206.83±95.24	5.93 ± 3.08

Notes: *In the responder group, LTG plasma concentrations differed significantly between patients receiving LTG monotherapy and those receiving LTG+VPA combination therapy ($P < 0.001$).

Laboratory Examinations

There were no significant differences between the two groups in terms of liver and kidney function, lipid profiles, or other related parameters (Supplement Tables 2 and 3). Compared with the non-responsive group, the responsive group showed significantly higher neutrophil percentage and serum uric acid levels, along with significantly lower absolute eosinophil count, platelet count, and hematocrit ($P < 0.05$). These findings suggest that liver and kidney functions associated with drug metabolism were comparable between the two groups, thereby ruling out hepatic or renal metabolic differences as major factors influencing LTG efficacy.

Therapeutic Drug Monitoring Analysis

Among the 141 patients, the mean LTG plasma concentration was (7.55 ± 4.57) mg/L in the responsive group and (4.53 ± 2.94) mg/L in the non-responsive group, with a statistically significant difference between the two groups ($P = 0.001$). The concentration range was 2.2–19.1 mg/L in the responsive group and 1.0–16.0 mg/L in the non-responsive group (Figure 1A). Receiver operating characteristic (ROC) curve analysis identified an optimal LTG plasma concentration

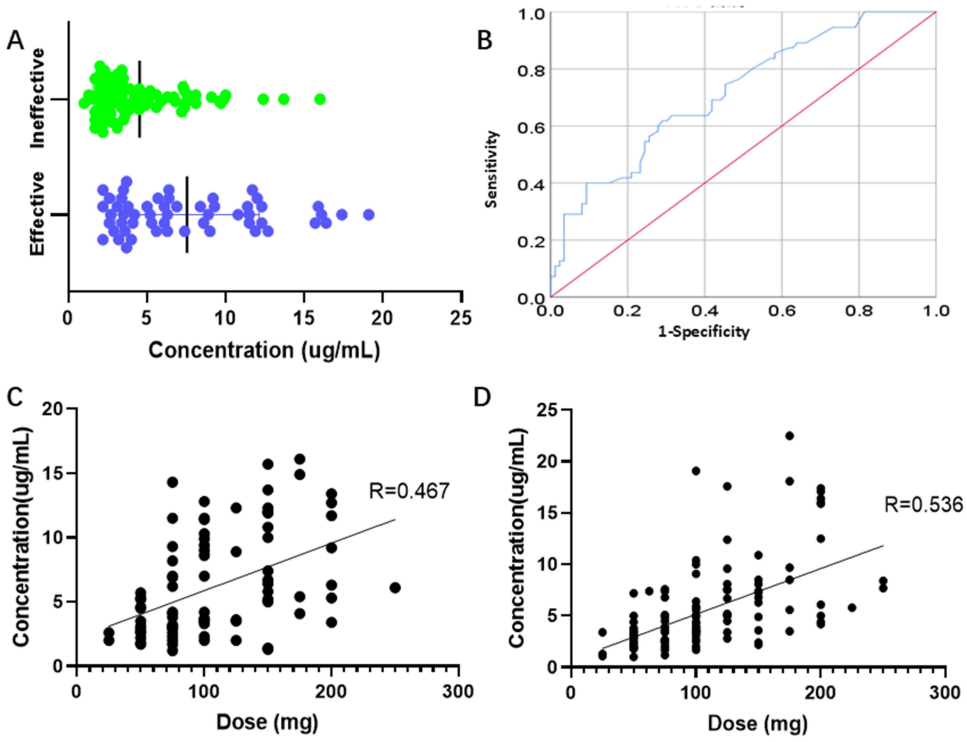


Figure 1 Analysis of LTG plasma concentrations and cut-off values. (**A**) Scatter plot showing the distribution of LTG plasma concentrations. (**B**) Receiver operating characteristic (ROC) curve used to determine the optimal cut-off value. (**C** and **D**) Scatter plots illustrating the correlation between plasma concentrations and daily dose in the effective group (C) and the ineffective group (D), respectively.

https://doi.org/10.2147/DDDT.S593268

cutoff value of 5.15 mg/L (Figure 1B). Patients with concentrations below 5.15 mg/L had a non-response rate of 74.1%, compared to 43.3% for those above this threshold. Pearson correlation analysis revealed a significant positive correlation between LTG plasma concentration and daily dose in both the responsive and non-responsive groups (Figure 1C and D).

Metabolomic Analysis of Epilepsy Treatment

Based on a LTG plasma concentration cutoff value of 5.15 mg/L, we selected high-concentration patients with LTG levels >5.15 mg/L, including 10 LTG responders and 10 LTG non-responders. The gender distribution (male/female) was 4/6 in both groups; mean ages were 34.60 ± 13.63 years and 40.00 ± 15.65 years, respectively; and LTG plasma concentrations were 9.45 ± 3.38 mg/L and 8.24 ± 2.13 mg/L, respectively (P = 0.352). No statistically significant differences were observed between the two groups of epilepsy patients in terms of complete blood count, liver function, renal function, or other clinical parameters.

Quality control (QC) analysis showed that the base peak chromatograms of QC samples exhibited high overlap, confirming that the LC-MS system stability met the required standards (Figure 2A). Using a dataset of 2,951 MS features detected by M1 Scan, a PLS-DA model was constructed. The results demonstrated clear separation between the responder and non-responder groups, with model parameters $R^2X = 0.609$, $R^2Y = 0.848$, and $Q^2 = 0.629$. The score plot further revealed distinct clustering of the two groups (Figure 2B). To validate model reliability, 200 permutation tests were performed. The original model's R^2 and Q^2 values were significantly higher than those from all permuted models, and the intercept of the regression line on the y-axis was below zero (Figure 2C). Potential differential metabolites contributing most to group separation were identified using an S-plot (Figure 2D).

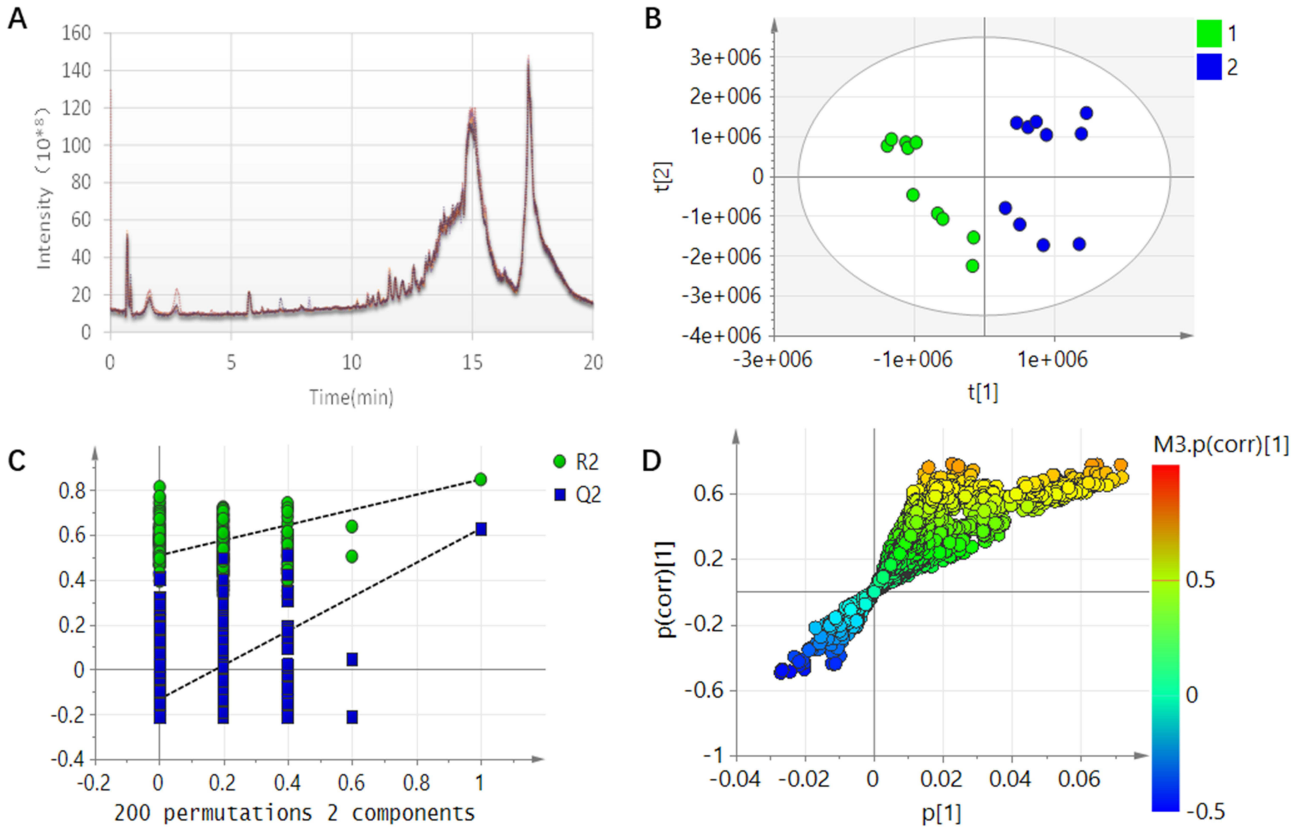


Figure 2 Metabolomic analysis of LTG responders versus non-responders based on the PLS-DA model. (**A**) LC-MS quality control (QC) chromatograms. (**B**) PLS-DA score plot showing clear separation between LTG responders and non-responders, indicating significant metabolic differences between the two groups. (**C**) Permutation test results (n = 200), demonstrating that the established model is statistically valid and not overfitted. (**D**) S-plot. Variables located at the two ends of the "S"-shaped curve represent potential biomarkers.

https://doi.org/10.2147/DDDT.S593268

Amino Acid Metabolic Profiling

Metabolite features with a VIP (Variable Importance in Projection) score > 1 and showing statistically significant differences in peak area between the LTG responder and non-responder groups were selected. These features were annotated by searching against the Human Metabolome Database (HMDB), the METLIN metabolite and tandem MS database, and the KEGG database. Ultimately, eight metabolites were identified: proline, 2-Octenoic acid, lysine, acetylenoxolone, leukotriene C4, erucamide, shearinine A, and 3,4-Dihydroxymandelic acid (Supplementary Table 4).

Based on these identified metabolites, we performed amino acid profiling in the two patient groups according to published method,[21] the typical mass chromatogram listed in Supplement Figure 2. The results revealed significant differences in amino acid metabolic profiles between LTG responders and non-responders. The most notable characteristics in the responder group were a significantly decreased level of L-cystine coupled with a marked elevation in proline levels (Table 3, $P < 0.05$).

Additionally, lysine levels were significantly lower in responders compared with non-responders. These metabolic alterations may indicate that patients in the responder group have a distinct amino acid metabolic status that could be related to redox balance and neuronal excitability. In contrast, the non-responder group exhibited an opposite pattern. Taken together, these exploratory findings suggest that specific amino acid metabolic pathways may be associated with the therapeutic efficacy of LTG and possibly other antiseizure medications; however, causality cannot be inferred from this study.

Prognostic Discriminant Model of RF

Based on the amino acid profiling results, we constructed two clinical datasets, one including amino acids and one excluding them, each comprising 20 responders and 20 non-responders with epilepsy. An RF model was developed using a cross-validation strategy that maintained consistent sample proportions between training and validation sets. The model was evaluated using 5-fold stratified sampling repeated five times. The results showed no further improvement in

Table 3 Differences in Amino Acid Profiles Between Effective Group and Ineffective Group to LTG Treatment

Amino Acid	Effective Group	Ineffective Group	P
Alanine	1.10±0.90	0.86±0.54	0.39
Arginine	0.88±0.73	1.12±0.86	0.34
Asparagine	0.89±0.49	1.11±0.65	0.22
Aspartic acid	0.94±0.88	1.06±0.61	0.64
Chloro-L-tyrosine	1.04±0.45	0.95±0.55	0.58
Citrulline	0.94±0.60	1.06±0.67	0.57
Cysteine	0.80±1.84	1.22±1.70	0.50
Cystine	0.49±0.40	1.05±1.00	0.03
Glutamic acid	0.92±0.57	1.08±0.62	0.42
Histidine	0.85±0.46	1.15±0.66	0.10
H-Tyr(3-NO2)-OH	1.06±0.47	0.93±0.55	0.42
Hydroxyproline	0.98±0.47	1.02±0.53	0.76
Leucine	0.94±0.54	1.06±0.64	0.54
Lysine	0.81±0.50	1.20±0.48	0.02
Methionine	0.98±0.46	1.02±0.55	0.79
Ornithine	0.89±0.55	1.11±0.65	0.25
Phenylalanine	1.00±0.31	1.00±0.43	0.95
Proline	1.23±0.82	0.77±0.47	0.04
Sarcosine	1.04±0.88	0.96±0.76	0.77
Serine	0.92±0.50	1.09±0.50	0.30
Threonine	1.09±0.71	0.91±0.47	0.35
Tryptophan	1.02±0.54	0.98±0.58	0.82
Tyrosine	0.95±0.46	1.05±0.51	0.48
Valine	0.97±0.58	1.03±0.51	0.72

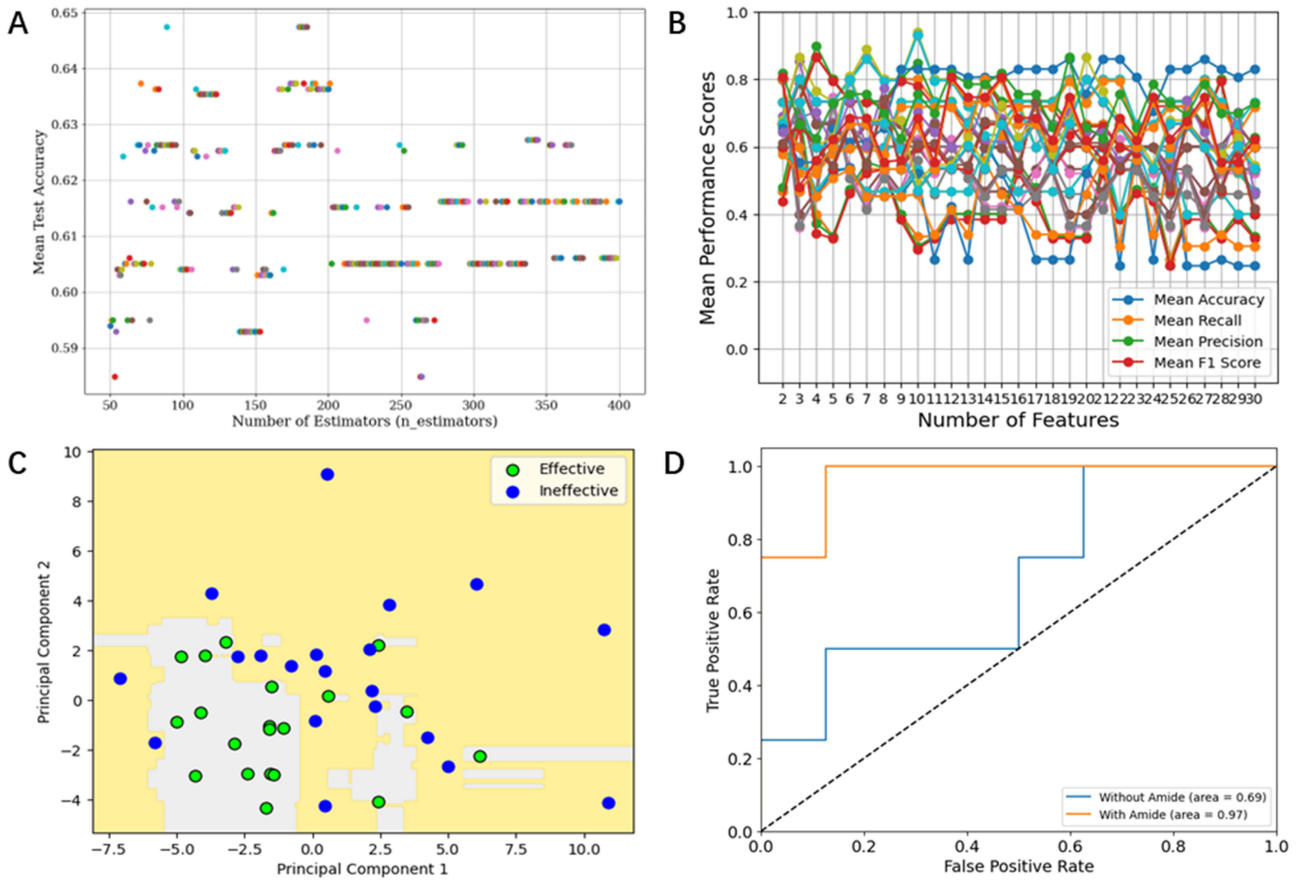


Figure 3 Classification results of random forest model and ROC analysis Parameter optimization (**A**) and feature selection evaluation of random forest (**B**), Classification results of random forest model (**C**) and ROC analysis with or without amino acids (**D**).

accuracy when the model used more than 100 trees or a maximum depth greater than 7; the parameter optimization results are shown in Figure 3A. The optimal parameters were "n_estimators = 89 and max_depth = 2". Regarding the effect of the number of features, the model did not show additional gains in predictive accuracy when more than two features were included; the evaluation of feature selection is presented in Figure 3B.

Using the established RF model, the classification results from the different datasets also showed that responders and non-responders could be completely separated, as illustrated in Figure 3C. The RF model was further used to perform ROC analysis on both the amino-acid-containing dataset and the dataset without amino acids, and the results are shown in Figure 3D. These findings indicate that the inclusion of amino acids substantially increases the ROC values, suggesting that the amino acids identified by metabolomic screening have potential value for early prediction of treatment response. Given the limited sample size and internal cross-validation design, the results of classification should be interpreted cautiously due to the potential risk of overfitting.

Discussion

By integrating therapeutic drug monitoring (TDM) with metabolomic analysis, this study investigated pharmacokinetic and metabolic factors that may underlie interindividual differences in LTG efficacy among patients with epilepsy.

We found that the mean plasma LTG concentration in responders (7.55 ± 4.57 mg/L) was significantly higher than that in non-responders (4.53 ± 2.94 mg/L), consistent with previous studies and supporting the clinical value of TDM in guiding individualized LTG therapy. Receiver operating characteristic (ROC) curve analysis suggested an LTG concentration of 5.15 mg/L as an optimal cut-off for distinguishing responders from non-responders in this cohort. Compared with the conventional therapeutic range recommended by the International League Against Epilepsy (ILAE) guidelines

https://doi.org/10.2147/DDDT.S593268

(2.5–15 mg/L), this finding may help refine clinical decision-making within the existing window, although it requires validation in larger, prospective cohorts.

Furthermore, the daily LTG dose was significantly higher in the responder group, which may reflect clinicians' proactive dose titration in response to suboptimal efficacy. This observation, together with the concentration–response relationship, underscores that achieving and maintaining an adequate plasma concentration is important for successful treatment.

Drug-combination analyses provided additional support for the concentration–efficacy relationship. Our data showed that VPA, a known inhibitor of UDP-glucuronosyltransferase (UGT) enzymes, was associated with significantly higher LTG plasma concentrations, in line with prior pharmacokinetic studies.[20] Notably, in the responder group, the LTG+VPA regimen achieved higher LTG concentrations and favorable clinical outcomes at a lower daily LTG dose compared with LTG monotherapy. These findings are consistent with the concept that VPA enhances LTG exposure by inhibiting its glucuronidation metabolism and may contribute to improved seizure control in some patients. However, given the retrospective and non-randomized design, potential confounding by indication cannot be excluded, and our results should be interpreted as supportive rather than definitive evidence for the clinical superiority of LTG+VPA over monotherapy.

Furthermore, it is crucial to consider the impact of exogenous estrogens on LTG pharmacokinetics. Combined oral contraceptives (OCPs) containing ethinylestradiol are known to be potent inducers of UGT1A4, the primary enzyme responsible for LTG glucuronidation. To maintain the internal validity of our study and ensure the homogeneity of the TDM and metabolomic data, we specifically excluded female patients using OCPs.

To move beyond conventional pharmacokinetic explanations and explore why some patients respond poorly despite comparable LTG concentrations, we employed an untargeted metabolomics approach in a subset of patients with LTG levels >5.15 mg/L. The PLS-DA model revealed distinct endogenous metabolic profiles between responders and non-responders. Through screening and targeted validation, we identified an amino acid-related metabolic signature associated with response, with proline, lysine, and cystine showing the most pronounced differences. Elevated proline levels in responders may have neurobiological implications; prior studies suggest that proline can influence GABAergic signaling or function as a compatible osmolyte during cellular stress, thereby stabilizing neuronal activity.[22,23] Conversely, lower lysine levels in responders might reflect differences in nitrogen or energy metabolism, although the precise mechanisms remain unclear.

Of particular interest, cystine (the oxidized dimer of cysteine) was markedly reduced in responders. The cystine/cysteine redox couple is an important component of intracellular redox buffering systems; lower cystine levels may indicate a more reduced intracellular environment, which could protect neurons from oxidative damage.[24]

Moreover, the cystine/glutamate antiporter (system x_c^-) imports extracellular cystine in exchange for glutamate.[25] One speculative explanation is that reduced cystine levels might reflect altered activity of system x_c^- sodium-channel blockade in seizure control. However, we did not directly measure oxidative-stress biomarkers or transporter function, and this hypothesis requires dedicated experimental validation.

Finally, we constructed random forest (RF) models to explore whether the inclusion of amino-acid variables could improve prediction of treatment response. In this small cohort (20 responders and 20 non-responders), the RF classifier showed excellent apparent performance, and the addition of amino acids increased ROC estimates. Nevertheless, these results are based on internal cross-validation only, without an independent test set, and the complete separation of responders and non-responders strongly suggests a risk of overfitting. Therefore, the RF models should be regarded as proof-of-concept rather than ready-to-use clinical tools, and external validation in larger datasets is essential.

To further strengthen our findings, future research should transition from a retrospective to a prospective design with a longer follow-up period. Additionally, multicenter studies with larger cohorts are warranted to validate the predictive value of the proline, lysine, and cystine profiles across diverse populations. Finally, functional studies are needed to elucidate the molecular mechanisms by which these amino acids influence redox balance and neuronal excitability, potentially uncovering new therapeutic targets for epilepsy.

Conclusion

This study confirms that LTG plasma concentration is a key predictor of its clinical efficacy, with an optimal individualized therapeutic threshold of 5.15 mg/L. It also clarifies the definitive role of valproic acid in enhancing LTG efficacy through

pharmacokinetic interactions. Metabolomic analysis further revealed that LTG response is associated with a distinct amino acid metabolic signature characterized by "elevated proline, and reduced lysine and cystine," suggesting that lower oxidative stress and a more stable neuronal metabolic environment constitute the intrinsic conditions for optimal LTG therapeutic outcomes.

Data Sharing Statement

The datasets generated and/or analyzed during this study are available upon legitimate request.

Funding

This work was supported by fund of Zhejiang Provincial Medical Association Project (YS2022-2-007), Medical Science and Technology Project of Zhejiang Province (2024KY406), health research project of National Natural Science Foundation of China (82104283).

Disclosure

These authors declare that there are no conflicts of interest regarding this work.

References

1. Kim YS, Kim MS, Park S. et al. Global, regional and national burden of epilepsy in children and adolescents, 1990–2021: a systematic analysis for the Global Burden of Disease Study 2021. *Eur J Clin Invest*. 2025:e70139. doi:10.1111/eci.70139.
2. Xue J, Li X, Zhao Y, et al. Global, regional, and national burden of idiopathic epilepsy in older adults, 1990–2021: a systematic analysis for the Global Burden of Disease Study 2021. *BMC Med*. 2025;23(1):443. doi:10.1186/s12916-025-04268-8
3. Sun T, Yu T, Liu P. Global, regional, and national burden of epilepsy, 1990–2021: a systematic analysis for the Global Burden of Disease Study in 2021. *Cost Eff Resour Alloc*. 2025;23(1):28. doi:10.1186/s12962-025-00635-7
4. International League Against Epilepsy Consortium on Complex E. GWAS meta-analysis of over 29,000 people with epilepsy identifies 26 risk loci and subtype-specific genetic architecture. *Nat Genet*. 2023;55(9):1471–1482. doi:10.1038/s41588-023-01485-w.
5. Barnard SN, Chen Z, Holmes M, et al. Treatment Response to Antiseizure Medications in People With Newly Diagnosed Focal Epilepsy. *JAMA Neurol*. 2025;82(10):1022–1030. doi:10.1001/jamaneurol.2025.2949
6. Bankole NDA, Dokponou YCH, De Koning R, et al. Epilepsy care and outcome in low- and middle-income countries: a scoping review. *J Neurosci Rural Pract*. 2024;15(1):8–15. doi:10.25259/JNRP_527_2023
7. Cerulli Irelli E, Cocchi E, Gesche J, et al. Lamotrigine vs levetiracetam in female patients of childbearing age with juvenile absence epilepsy: a Bayesian reanalysis. *Epilepsia*. 2024;65(10):2897–2908. doi:10.1111/epi.18087
8. Watanabe S, Enokizono T, Nishimura M, et al. Valproate and lamotrigine combination therapy in children with drug-resistant focal epilepsy: an observational analysis focusing on neuroimaging abnormalities. *Brain Dev*. 2025;47(4):104395. doi:10.1016/j.braindev.2025.104395
9. Marson AG, Al-Kharusi AM, Alwaidh M, et al. The SANAD study of effectiveness of valproate, lamotrigine, or topiramate for generalised and unclassifiable epilepsy: an unblinded randomised controlled trial. *Lancet*. 2007;369(9566):1016–1026. doi:10.1016/S0140-6736(07)60461-9
10. Glauser T, Ben-Menachem E, Bourgeois B, et al. Updated ILAE evidence review of antiepileptic drug efficacy and effectiveness as initial monotherapy for epileptic seizures and syndromes. *Epilepsia*. 2013;54(3):551–563. doi:10.1111/epi.12074
11. Wang ZZ, Zhang YF, Huang WC, et al. Effects of Comedication and Genetic Factors on the Population Pharmacokinetics of Lamotrigine: a Prospective Analysis in Chinese Patients With Epilepsy. *Front Pharmacol*. 2019;10:832. doi:10.3389/fphar.2019.00832
12. Petrenaite V, Ohman I, Jantzen FPT, Ekstrom L. Effect of UGT1A4, UGT2B7, UGT2B15, UGT2B17 and ABC1B polymorphisms on lamotrigine metabolism in Danish patients. *Epilepsy Res*. 2022;182:106897. doi:10.1016/j.eplepsyres.2022.106897
13. Morris RG, Black AB, Lam E, Westley IS. Clinical study of lamotrigine and valproic acid in patients with epilepsy: using a drug interaction to advantage? *Ther Drug Monit*. 2000;22(6):656–660. doi:10.1097/00007691-200012000-00003
14. Patsalos PN, Berry DJ, Bourgeois BF, et al. Antiepileptic drugs--best practice guidelines for therapeutic drug monitoring: a position paper by the subcommission on therapeutic drug monitoring, ILAE Commission on Therapeutic Strategies. *Epilepsia*. 2008;49(7):1239–1276. doi:10.1111/j.1528-1167.2008.01561.x
15. Sondergaard Khinchi M, Nielsen KA, Dahl M, Wolf P. Lamotrigine therapeutic thresholds. *Seizure*. 2008;17(5):391–395. doi:10.1016/j.seizure.2007.11.023
16. Li XW, He H, Liu YF, et al. Effectiveness and safety assessment of lamotrigine monotherapy for treatment of epilepsy. *Eur Rev Med Pharmacol Sci*. 2012;16(10):1409–1413.
17. Marson A, Burnside G, Appleton R, et al. The SANAD II study of the effectiveness and cost-effectiveness of levetiracetam, zonisamide, or lamotrigine for newly diagnosed focal epilepsy: an open-label, non-inferiority, multicentre, Phase 4, randomised controlled trial. *Lancet*. 2021;397(10282):1363–1374. doi:10.1016/S0140-6736(21)00247-6
18. Wang X, Chen Z, Ke X, Wang Y, Hu L, Tang C. Comparison of HPLC-DAD and UPLC-MS/MS in Monitoring Serum Concentration of Lamotrigine. *Curr Pharml Anal*. 2022;18(5):449–454. doi:10.2174/1573412917666210215150712
19. Tong S, Wu S, Wu J, et al. Random forest classification of four unsaturated fatty acids for infection diagnosis and prognosis in female Chinese patients. *Ann Med Surg Lond*. 2025;87(8):4841–4847. doi:10.1097/MS9.0000000000003570
20. Liu L, Zhao L, Wang Q, Qiu F, Wu X, Ma Y. Influence of valproic acid concentration and polymorphism of UGT1A4*3, UGT2B7 −161C > T and UGT2B7*2 on serum concentration of lamotrigine in Chinese epileptic children. *Eur J Clin Pharmacol*. 2015;71(11):1341–1347. doi:10.1007/s00228-015-1925-9

21. Deng M, Lin F, Zhou C, et al. Determination of 27 amino acids' levels in seminal plasma of asthenospermia and oligospermia patients and diagnostic value analysis. *J Pharm Biomed Anal*. 2020;184:113211. doi:10.1016/j.jpba.2020.113211
22. Kan ASH, Kusay AS, Mohammadi NA, et al. Understanding paralogous epilepsy-associated GABA(A) receptor variants: clinical implications, mechanisms, and potential pitfalls. *Proc Natl Acad Sci U S A*. 2024;121(50):e2413011121. doi:10.1073/pnas.2413011121
23. Yang L, Cai X, Zhou J, et al. STE20/SPS1-related proline/alanine-rich kinase is involved in plasticity of GABA signaling function in a mouse model of acquired epilepsy. *PLoS One*. 2013;8(9):e74614. doi:10.1371/journal.pone.0074614
24. Alcoreza OB, Patel DC, Tewari BP, Sontheimer H. Dysregulation of Ambient Glutamate and Glutamate Receptors in Epilepsy: an Astrocytic Perspective. *Front Neurol*. 2021;12:652159. doi:10.3389/fneur.2021.652159
25. Leclercq K, Liefferinge JV, Albertini G, et al. Anticonvulsant and antiepileptogenic effects of system xc- inactivation in chronic epilepsy models. *Epilepsia*. 2019;60(7):1412–1423. doi:10.1111/epi.16055